American Chemistry Council *Good Chemistry Makes It Possible*

January 3, 2002

8EHQ-0102-15040

Ms. Christine Todd Whitman
Administrator
U. S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

Dear Ms. Whitman:

The American Chemistry Council (Council) makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following final report that the Council's Brominated Flame Retardant Industry Panel (BFRIP) recently conducted is enclosed:

- Hexabromocyclododecane (HBCD): A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats.

This report does not include confidential information.

If you have any questions, please contact Wendy K. Sherman, the BFRIP Manager, at 703/741-5639 or via email [wendy_sherman@americanchemistry.com].

Sincerely yours,

Elizabeth Festa Watson
Managing Director, CHEMSTAR

Enclosure (4 volumes)

Contain NO CBI

8EHQ-02-15040

88020000026

Responsible Care®

MR-54001

1300 Wilson Boulevard, Arlington, VA 22209 • Tel 703-741-5000 • Fax 703-741-6000 • http://www.americanchemistry.com

FINAL REPORT

Volume 1 of 4
(Text and Summary Tables 1-68)

STUDY TITLE

A 90-DAY ORAL (GAVAGE) TOXICITY STUDY OF HBCD IN RATS

STUDY DIRECTOR

Christopher P. Chengelis, Ph.D., D.A.B.T.

STUDY INITIATED ON

March 2, 2000

STUDY COMPLETION DATE

December 14, 2001

PERFORMING LABORATORY

WIL Research Laboratories, Inc.
1407 George Road
Ashland, Ohio 44805-9281

LABORATORY STUDY NUMBER

WIL-186012

SPONSOR

Chemical Manufacturers Association
Brominated Flame Retardant Industry Panel (BFRIP)
1300 Wilson Blvd.
Arlington, VA 22209

A 90-Day Oral (Gavage) Toxicity
Study of HBCD in Rats

I. SUMMARY

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat (mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.

Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T_3, T_4 and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to initiation of dosing and during study weeks 12 and 15. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to initiation of dosing, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was

performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs and/or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs, prostate glands and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical comparisons by sex and treatment day were made between the control and treated animals where indicated ($p<0.05$).

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article-related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were generally secondary to the inducing effects on the liver or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p<0.05$ or $p<0.01$) test article-related clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p<0.01$). Thyroxine (T_4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p<0.05$ or $p<0.01$). There were no corresponding statistical effects on T_3 and TSH. While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period; furthermore, these serum albumin and gamma glutamyltransferase increases were probably secondary to the increases in liver weight. The increases in serum chloride were probably secondary to be presence of free bromide in the test article preparation which interfered with the chloride determination methodology. The decrease in T_4, which was also reversible, was also probably secondary to increased liver weight (secondary to microsomal enzyme induction, known to cause increased metabolism and clearance of T_4 in the rat).

The incidence of observations noted at gross necropsy was low and there was no evidence of frank organ damage. On histopathologic examination of tissues, relatively mild findings occurred in both the control and treated groups. Potential test article-related histologic changes were identified in the liver and thyroid glands but these would not be considered indicative of frank toxicity. These organs were examined microscopically in all groups at both necropsies. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Minimal hepatocellular

vacuolation was also detected in females in the control and test article treated groups without a clear dose response (3 to 6/10 animals per group) but, mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10 and 3/10 females in the 300 and 1000 mg/kg/day groups, respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females and 1/10 males in the 1000 mg/kg/day group. The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p<0.05$ or $p<0.01$). The increases in liver weight were a result of a microsomal enzyme inducing effect[1] and were not typically considered indicative of toxicity in absence of frank organ damage. The reversible histologic changes (vacuolation and hypertrophy) are often found to accompany increased liver weight caused by liver enzyme induction. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects. The histologic changes in the thyroid had also nearly completely resolved except in the 1000 mg/kg/day group females, where partial recovery occurred.

Increases in mean prostate weight were noted in the 1000 mg/kg/day group males at the primary necropsy. However, the increases in prostate weight were probably not of toxicological significance since the increases did not persist to the

recovery period, there were no correlating histologic findings and no change in sperm production.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta which is in contrast to the test article composition (gamma>>alpha>beta). Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The additional findings observed at 1000 mg/kg/day (increased gamma glutamyltransferase and additional increases in the size of the liver and prostate), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, probably of limited, if any, toxicologic significance. On this basis the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day.